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PITYROSPORUM ORBICULARE: INCIDENCE AND DISTRIBUTION ON CLINICALLY NORMAL SKIN

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SUMMARY.—In a microscopical and cultural survey of clinically normal skin of patients attending a skin clinic with minor complaints *P. orbiculare* was found to be present on the scalp in 74%. *P. ovale* was numerically the dominant species in this site but on the trunk *P. orbiculare* was nearly always the more numerous yeast and was present in more than 90% of subjects. The occasional finding of a few rod or filamentous forms similar to those seen in pityriasis versicolor was noted. This phenomenon was associated with a greater tendency than usual for the yeast to form germ tubes in culture. The significance of this is discussed.

Pityrosporum orbiculare was the name given by Gordon (1951) to the spherical lipophilic yeast which he cultured from the lesions of pityriasis versicolor. Unlike the fungus seen on direct examination of affected skin and known as *Malassezia furfur*, the cultured fungus showed only yeast forms with no tendency to develop filaments. Much subsequent work has been devoted to the task of elucidating the relationships between *P. orbiculare* and *M. furfur* and it is now generally thought that they represent different phases of the same organism.

Gordon also reported the finding of spherical budding yeasts on 7 of 8 normal subjects and that on culture he had isolated *P. orbiculare* from 2 of them. Flask shaped or spherical budding yeasts had been seen occasionally before but were generally dismissed as a variant of the more familiar bottle shaped and oval yeasts known as *Pityrosporum ovale*. This aspect of Gordon's work has been largely neglected and the importance of *P. orbiculare* as a member of the normal skin flora has not been established. For this reason, and in an attempt to provide a base line for a companion study of lipophilic yeasts in patients with pityriasis versicolor, the investigation reported here was undertaken.

METHODS AND MATERIALS

A hundred subjects were studied. They were all white Caucasoids living in England. A few were normal volunteers but most were patients attending the dermatology outpatient department. Consecutive patients at selected clinics with trivial complaints or lesions of limited extent were investigated. (See footnote * for clinical diagnoses.) This method of selecting patients resulted in a preponderance of female subjects. All sites sampled were clinically normal and had not been affected in the past as far as the patient

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* Diagnoses of patients investigated:—Acne, Acrocyanosis, Acrosclerosis, Atopic dermatitis, Basal cell carcinoma, Berloque dermatitis, Chloasma, Chondrodermatitis helices, Contact dermatitis, Discoid eczema, Discoid lupus erythematosus, Eczema—unclassified, Erythema multiforme, Furunculosis, Granuloma fissuratum, Herpes simplex, Keloid, Keratoacanthoma, Leg ulcer, Lichen planus, Mastocytosis, Nail dystrophy, Parapsoriasis, Perioral dermatitis, Pigmented naevi, Pompholyx, Prurigo, Pruritus ani, Psoriasis, Rosacea, Sebaceous cysts, Squamous cell epithelioma, Thrombophlebitis, Tinea pedis, Urticaria, Virus warts.

could recall. No sites treated with topical applications were sampled and no patients on systemic therapy with cortico-steroids, immunosuppressives or antibiotics were included. All cases of seborrhoeic dermatitis of any pattern were excluded, but mild acne was not, provided the criteria above were satisfied. A few psoriatics with very limited lesions were included. Scrapings were taken using a fresh sterile scalpel blade for each site sampled.

The scalp (100 subjects) was sampled at approx. 3 or 4 cm. inside the hair line in the frontal region away from the midline. Where male pattern alopecia was encountered a more lateral site that was still hair bearing was taken. The chest (50 subjects) was sampled in the midline just below the angle of the sternum, the back (10 subjects) in the interscapular region. All specimens were examined microscopically using the Parker Ink/KOH technique (Cohen, 1954). It was found to give excellent results provided at least 2 hr. was allowed for clearing and staining. Many specimens were re-examined the following day having been covered overnight with a petri dish lid. The amount of material taken was very variable but the area scanned microscopically was generally about 10 sq. mm. *P. orbiculare* was recorded as present if thick walled, spherical budding forms were seen. The presence of clusters of this species was noted and a search made for rods or filaments such as are seen in pityriasis versicolor. All oval forms were recorded as *P. ovale*.

Cultures

The medium used was 3% malt extract (Oxoid) pH 5.4 approximately, containing penicillin, streptomycin and actidione. Specimens were inoculated into the level surface of the medium in small 5 mm. screw top bottles and covered with a thin layer of sterile olive oil. They were cultured at 30°C. Isolates were identified at approximately 7 days. If no growth had occurred at that time they were retained and checked at approximately 14 days. The presence of germ tubes was noted and a search made for any attempt at hypha formation. The precise lipid requirements of isolates of *P. orbiculare* were not investigated but in a sample of cases lipid dependence in sub-culture was studied. All these isolates showed dependence on lipid (olive oil) and negligible growth without it.

RESULTS

See Table I

The Scalp.—In all, 74 subjects were judged to carry *P. orbiculare*. In 7 subjects there was marked clustering of *P. orbiculare* and in 5, rods or filaments were noticed. *P. ovale* was carried by 97 subjects. In the 3 subjects not apparently carrying *P. ovale*, *P. orbiculare* was present in culture. A further 3 subjects were judged to have a definite predominance of *P. orbiculare*. Most of the other 94 subjects had a definite preponderance of oval forms.

Chest.—*P. orbiculare* was carried by 46 subjects (92%). Clusters of *P. orbiculare* were present in 7 cases (14%), filaments were found in 4 cases (8%). A total of 46 subjects (92%) appeared to carry *P. ovale* on the chest. In 33 (66%) *P. orbiculare* predominated. In 9 subjects the proportions of the two yeasts were approximately equal and in 7 *P. ovale* was more numerous than *P. orbiculare*. One subject yielded neither organism on culture and no yeasts were seen on microscopy.

Back.—*P. orbiculare* was carried by all 10 subjects, clusters were seen in 5 and filaments in 2 subjects. *P. ovale* was found on 6 subjects. *P. orbiculare* predominated in 7 out of 10 subjects and in the other 3 the two yeasts were present in almost equal numbers.

TABLE I.

Scalp

100 Subjects. 37 Males. 63 Females. Ages 6 months-93 years.

	Direct examination		Culture		Total +ve	Total -ve
	+ve	-ve	+ve	-ve		
<i>P. orbiculare</i>	68	32	54	14	74 (74%)	26 (26%)
			6	26		
<i>P. ovale</i>	97	3	51	46	97 (97%)	3 (3%)
			0	3		

Chest

50 Subjects. 11 Males. 39 Females. Ages 15-74 years.

	Direct examination		Culture		Total +ve	Total -ve
	+ve	-ve	+ve	-ve		
<i>P. orbiculare</i>	45	5	42	3	46 (92%)	4 (8%)
			1	4		
<i>P. ovale</i>	38	12	26	12	46 (92%)	4 (8%)
			8	4		

Back

10 subjects. 3 Males. 7 Females. Ages 15-52 years.

	Direct examination		Culture		Total +ve	Total -ve
	+ve	-ve	+ve	-ve		
<i>P. orbiculare</i>	10	0	9	1	10 (100%)	0 (0%)
			0	0		
<i>P. ovale</i>	6	4	5	1	8 (80%)	2 (20%)
			2	2		

Other Sites.—Most of these results come from 2 normal subjects with the addition of further information from a handful of others, where 1 or 2 sites only were scraped. Diagram I shows sites where *P. orbiculare* was seen on microscopy. The number of organisms seen was generally small on the limbs and higher on the trunk, the face and the flexures where the levels were comparable with those on the chest or back. Most peripheral sites were sterile on culture but the organism was isolated from the following regions—forehead, the face (naso-genial fold), the shoulder, the abdomen, the lower back, the axilla, the groin, the forearm (flexural surface) and the palms. *P. ovale* appeared to be present in large numbers on the face and in small numbers on all the other sites sampled.

Germ tube formation.—On the scalp, chest and back there were 105 sites in 73 subjects which were positive on microscopy and culture for *P. orbiculare*. Of these sites 14 gave cultures showing germ tube formation. Of 10 sites (on 8 subjects) which showed filament formation on microscopy, 6 (60%) yielded isolates forming germ tubes in culture. Of the 95 sites (in 68 subjects) which did not show filament formation only 8 (8.4%) yielded isolates forming germ tubes. Considering subjects rather than sites 8 showed filament formation on 1 or more areas. Of these 5 (62.5%) yielded at least 1 isolate from 1 site or another which formed germ tubes. Of 65 subjects not showing filament formation 7 (10.8%) gave isolates which produced germ tubes on culture. These associations are statistically highly significant. ($p < 0.001$.)

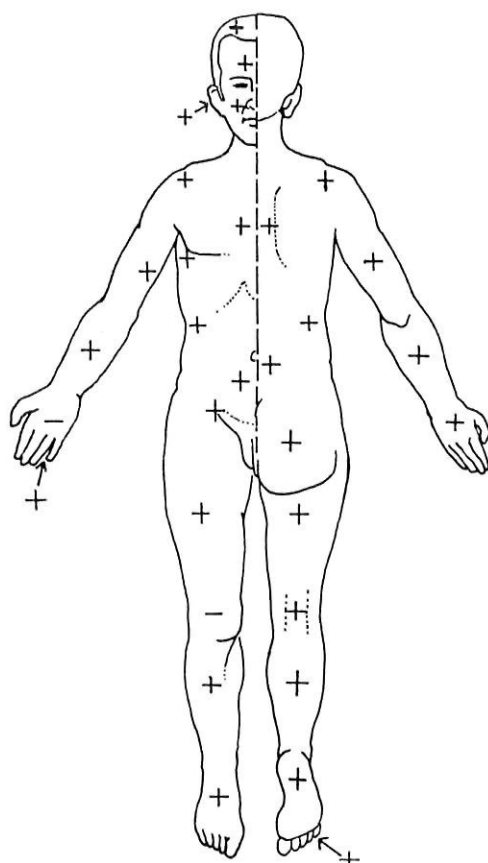


FIG. 1.—Distribution of *Pityrosporum orbiculare*. Direct examination: + = *P. orbiculare* seen; — = *P. orbiculare* not seen.

DISCUSSION

Following Panja's lead in using vegetable oils, Gordon (1951*a*, 1951*b*) successfully isolated a spherical lipophilic yeast from 13 of 18 cases of pityriasis versicolor. This he called *Pityrosporum orbiculare*. In spite of his failure to produce lesions of pityriasis versicolor by inoculation of *P. orbiculare* into normal subjects and in spite of the lack of the rod formation in culture, this aspect of Gordon's work received much attention. Burke (1961) and Keddie and her co-workers (Sternberg and Keddie, 1961; Keddie, 1966) provided further evidence suggesting that *P. orbiculare* does in fact represent the yeast phase of *M. furfur*. The final proof, successful production *in vitro* of *M. furfur*-like forms from *P. orbiculare* cultures, awaits the discovery of a suitable medium.

The other aspect of Gordon's report, his observation that *P. orbiculare* was probably part of the normal flora of the skin in yeast form excited much less interest. Martin Scott (1952) using a medium intended primarily for the isolation of *P. ovale* appears not to have isolated *P. orbiculare* from any normal subjects. DiMenna

(1954) in a survey which included the microscopical examination of scrapings from 84 normal scalps did not differentiate between the 2 forms of lipophilic yeast but did however notice on 2 subjects the presence of rod like forms similar to those of *Malassezia furfur*. She reported no successful cultures. Marples (1965) in her account of the frequency and distribution of lipophilic yeasts on the normal skin relied heavily on the surveys of Spoor *et al.* (1954) and Martin Scott. She concluded that "our knowledge of the ecology of *P. ovale* is fragmentary". In the case of *P. orbiculare* she had to rely largely on interpolation of the work done on pityriasis versicolor and she was unable to quote a single survey of *P. orbiculare* on normal skin. More recently Alexander (1967) has recorded the presence of *P. orbiculare* on normal scalps in a survey primarily directed towards the relationship between the yeast flora on the scalp, dandruff and alopecia. She studied 86 patients with dandruff and obviously accepts that *P. orbiculare* and *P. ovale* are both members of the normal flora of this region but she does not record the frequency with which she found either organism nor which one predominated.

In clinical accounts of pityriasis versicolor the acceptance of the probable identity of *P. orbiculare* and *M. furfur* is now general but the place of *P. orbiculare* as a member of the normal flora tends to be ignored. Wilson and Plunkett (1965) while joining in this general acceptance of the identity of these two organisms can write "probably all human beings are at some time or other contacted by the spores of *M. furfur*". Marples does go further and accepts that the organism is probably a member of the normal flora at least in some subjects. The patients sampled in this survey are of course not completely normal. There is little reason to expect that patients with warts, basal cell carcinomata, leg ulcers etc. should differ significantly from the general healthy population in respect of the lipophilic yeasts they carry on their skin and comparisons between the various diagnostic categories show no apparent differences. The small number of males in the survey is unfortunate but as far as can be seen there are no gross differences between the sexes in respect of their lipophilic yeast flora.

The methods used require little comment. Most earlier surveys used Sabouraud's medium or Mycotel agar rather than 3% malt extract. In the present study a little more olive oil appears to have been used than is common. The choice of 30° C. as the incubation temperature is largely accidental. Earlier work with pityriasis versicolor was done for convenience in an incubator used for general work and set at 28° C. When a second incubator became available the temperature was raised a little with perhaps slightly improved results, but it was not felt necessary to use the generally preferred temperature of 37° for this survey.

The results reported here show that *P. orbiculare* is a common member of the normal cutaneous flora in man and is probably present in nearly all the population. Its headquarters, to use Marples' terminology, seems to be on the trunk, flexures and the face and to a lesser extent in the scalp. Most sites of the body however seem to support a small population of this organism. The distribution of both *P. orbiculare* and *P. ovale* on the skin is patchy. Because of this the determination of the predominant organism in any site may be inaccurate. It would however seem that *P. ovale* is undoubtedly the predominant yeast on the scalp and *P. orbiculare* is nearly always present in greater numbers than *P. ovale* on the trunk. The importance of *P. orbiculare* in the flexures requires further study.

The finding on 8% of subjects of rods of size and shape similar to those of *Malassezia furfur* is of interest in view of DiMenna's earlier report. Where these

rods were seen, there were always a few, and sometimes many, round yeasts (*P. orbiculare*), and on culture of these sites all but 1 of them yielded *P. orbiculare*. This finding would seem to add weight to the evidence already heavy in favour of the identity of *P. orbiculare* and *Malassezia furfur*. Perhaps these sites showing rod formation should be considered as subclinical or microscopic pityriasis versicolor. Burke (1961) reports that germ tubes are formed by *P. orbiculare* in culture fairly commonly but in this survey they were only seen occasionally. The finding that germ tubes were very much more common in the isolates obtained from sites where filament formation had been seen, than sites where it had not, and that they were also much more common in isolates obtained from patients who showed filament formation somewhere on the body is of interest. It is tentatively suggested that it might imply that some strains of *P. orbiculare* show a greater tendency than others to yeast/mycelial transformation and that these strains might be more likely to produce pityriasis versicolor given a susceptible subject. This is not at variance with Burke's observation that germ tube formation is common, as most if not all of her isolates appear to have come from subjects suffering from pityriasis versicolor.

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